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File: USPT

Nov 21, 2000

US-PAT-NO: 6149919DOCUMENT-IDENTIFIER: US 6149919 A

TITLE: Immunogenic detoxified mutants of cholera toxin and of the toxin LT, their preparation and their use for the preparation of vaccines

DATE-ISSUED: November 21, 2000

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Domenighini; Mario	Quercegrossa			IT
Rappuoli; Rino	Quercegrossa			IT
Pizza; Mariagrazia	Siena			IT
Hol; Wim	Seattle	WA		

US-CL-CURRENT: 424/236.1; 424/184.1, 424/234.1, 424/240.1, 424/241.1, 424/257.1, 424/261.1, 435/69.3

## CLAIMS:

We claim:

1. An immunogenic detoxified protein comprising an amino acid sequence of subunit A of a cholera toxin or a fragment thereof or an amino acid sequence of subunit A of an Escherichia coli heat labile toxin or a fragment thereof comprising one or more of the following amino acid replacements at or in positions corresponding to amino acids in the sequence of subunit A of mature cholera toxin: Val-53-Asp, Val-53-Glu, Val-53-Tyr, Ser-63-Lys, Val-97-Lys, Val-97-Tyr, Tyr-104-Lys, Tyr-104-Asp, Tyr-104-Ser, and Pro-106-Ser, wherein said fragment comprises from 3 to about 5 amino acids of at least one of the regions containing one of the amino acids to be replaced.
2. An immunogenic composition comprising an immunogenic detoxified protein according to claim 1 and a pharmaceutically acceptable carrier.
3. A vaccine comprising an amino acid sequence of subunit A of a cholera toxin or an amino acid sequence of subunit A of an Escherichia coli heat labile toxin comprising one or more of the following amino acid replacements at or in positions corresponding to amino acids in the sequence of subunit A of mature cholera toxin: Val-53-Asp, Val-53-Glu, Val-53-Tyr, Ser-63-Lys, Val-97-Lys, Val-97-Tyr, Tyr-104-Lys, Tyr-104-Asp, Tyr-104-Ser, and Pro-106-Ser, wherein said fragment comprises from about 3 to about 5 amino acids of at least one of the regions containing one of the amino acids to be replaced.
4. A method of vaccinating a mammal against Vibrio cholerae or an enterotoxigenic strain of Escherichia coli comprising administering an

immunologically effective amount of an immunogenic detoxified protein according to claim 1.

5. A process for the formulation of a vaccine comprising bringing an immunogenic detoxified protein according to claim 1 into association with a pharmaceutically acceptable carrier.

6. A process for the formulation of a vaccine comprising bringing an immunogenic detoxified protein according to claim 1 into association with an adjuvant.

7. An immunogenic detoxified protein comprising an amino acid sequence of subunit A of a cholera toxin or a fragment thereof or an acid sequence of subunit A of an Escherichia coli heat labile toxin or a fragment thereof wherein the position corresponding to Ser-63 in the sequence of subunit A of mature cholera toxin is replaced with lysine, wherein said fragment comprises from about 3 to about 5 amino acids of at least one of the regions containing one of the amino acids to be replaced.

8. An immunogenic detoxified protein comprising an amino acid sequence of subunit A of a cholera toxin or a fragment thereof or an amino acid sequence of subunit A of an Escherichia coli heat labile toxin or a fragment thereof wherein the position corresponding to Pro-106 in the sequence of subunit A of mature cholera toxin is replaced with serine, wherein said fragment comprises from about 3 to about 5 amino acids of at least one of the regions containing one of the amino acids to be replaced.

9. A vaccine composition according to claim 3 further comprising an adjuvant.

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Domenighini; Mario	Quercegrossa			IT
Rappuoli; Rino	Quercegrossa			IT
Pizza; Mariagrazia	Siena			IT
Hol; Wim	Seattle	WA		

## ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
Biocine S.p.A.	Siena			IT	03

APPL-NO: 08/823120 [PALM]

DATE FILED: March 25, 1997

## PARENT-CASE:

This application is a continuation of application Ser. No. 08/256,003, filed Nov. 11, 1994, which is a filing under 35 U.S.C. 371 of PCT/EP92/03016, filed Dec. 30, 1992 abandoned; and which is a filing from parent Italian patent application M191 A 003513, filed Dec. 31, 1991.

## FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
IT	M191A03513	December 31, 1991

INT-CL-ISSUED: [07] A61K 39/02, A61K 39/108, A61K 39/106, C12P 21/06

## INT-CL-CURRENT:

TYPE IPC	DATE
CIPS <u>C07 K 14/28</u>	20060101
CIPN <u>A61 K 38/00</u>	20060101
CIPN <u>A61 K 39/00</u>	20060101
CIPS <u>C07 K 14/245</u>	20060101
CIPS <u>C07 K 14/195</u>	20060101

US-CL-ISSUED: 424/236.1; 424/184.1, 424/234.1, 424/240.1, 424/241.1, 424/257.1, 424/261.1, 435/69.3, 435/172.3

US-CL-CURRENT: 424/236.1; 424/184.1, 424/234.1, 424/240.1, 424/241.1, 424/257.1, 424/261.1, 435/69.3

FIELD-OF-CLASSIFICATION-SEARCH: 424/240.1, 424/241.1, 424/257.1, 424/261.1, 424/184.1, 424/234.1, 424/236.1, 435/69.3, 435/172.3

See application file for complete search history.

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

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	PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
<input type="checkbox"/>	<u>4666837</u>	May 1987	Harford et al.	435/68
<input type="checkbox"/>	<u>4935364</u>	June 1990	Kaper et al.	435/172.3

FOREIGN PATENT DOCUMENTS

FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	CLASS
WO 92/19265	November 1992	WO	
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ART-UNIT: 161

PRIMARY-EXAMINER: Housel; James C.

ASSISTANT-EXAMINER: Portner; Virginia

ATTY-AGENT-FIRM: Trujillo; Doreen Y. Harbin; Alisa A. Blackburn; Robert P.

#### ABSTRACT:

An immunogenic detoxified protein comprising the amino acid sequence of subunit A of cholera toxin (CT-A) or subunit A of an Escherichia coli heat labile toxin (LT-A) or a fragment thereof wherein one or more amino acids at, or in positions corresponding to Val-53, Ser-63, Val-97, Tyr-104 or Pro-106 are replaced with another amino acid or deleted. Examples of specific replacements include Val-53-Asp, Val-53-Glu, Val-53-Tyr, Ser-63-Lys, Val-97-Lys, Val-97-Tyr, Tyr-104-Lys, Tyr-104-Asp, Tyr-104-Ser, Pro-106-Ser. The immunogenic detoxified protein is useful as vaccine for Vibrio cholerae or an enterotoxigenic strain of Escherichia coli and is produced by recombinant DNA means by site-directed mutagenesis.

9 Claims, 4 Drawing figures

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